

TREPONEMA PALLIDUM IMMUNE-ADHERENCE AND HAEMAGGLUTINATION*†

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According to Nelson (1953), "the immune-adherence phenomenon" is concerned with the specific attachment which occurs between erythrocytes and micro-organisms which are sensitized by antibody (Ab) and Complement (C'). Measurements of immune-adherence (IA) may be performed by counting the *Treponema pallidum* remaining in the fluid phase of reaction mixtures containing *T. pallidum*, Ab, C', and erythrocytes. The counting of *T. pallidum* under dark-field examination with high dry magnification is liable to error. Errors of counting are mainly due to the difficulty of placing between slide and coverslip an accurately calibrated volume of fluid mixture, and also from the unequal distribution of the *T. pallidum*. Following Nelson's suggestions, we have studied a new procedure of IA by which readings are made easier and more reliable. This makes use of the haemagglutination induced by IA. This method shows the agglutination of erythrocytes attached to *T. pallidum* sensitized with specific Ab and C'. The reading is similar to other haemagglutination tests.

Materials and Methods

The reagents used in the new technique are exactly the same as those used in the regular TPIA method.

(i) *Treponeme* suspension (Nichols strain) is prepared in the regular TPI medium or in a simplified medium with buffered saline and bovine albumin (0.5 per cent.).

(ii) *Treponemes* harvested from an early orchitis are killed by heating for 30 min. at 56° C. They are absorbed with human erythrocytes and stored at + 5° C. Streptomycin (1/1000) is added to avoid bacterial contamination. The *T. pallidum* suspension is calibrated to contain about 100 organisms per field with high dry magnification.

(iii) Complement is obtained from fresh human serum from a blood donor (Group O).

(iv) Guinea-pig C' may also be used after absorption with human erythrocytes.

(v) Sera to be tested are heated to 56° C. for 30 min.

(vi) Human erythrocytes are obtained from a blood donor, Group O.

(vii) Cells are washed four times with saline and stored in Alsever's solution. Erythrocytes are washed four times before use and resuspended at 2 per cent. concentration in regular TPI medium or in fresh saline.

Technique of Haemagglutination Test

Haemagglutination tests were carried out in 25 × 100-mm. tubes in a total test volume of 1.25 ml., 1.00 ml. antigen, 0.05 ml. C', and 0.10 ml. diluted serum (1/5). The reaction mixtures were incubated in a water bath for 15 min. at 37° C., and 0.10 ml. 2 per cent. erythrocytes was added. The reaction mixtures were shaken and reincubated for 10 min. at 37° C. Then, after gentle shaking, the mixtures were allowed to sediment for 1 hr at 37° C. or at room temperature. The readings were made after 60 or 90 min., even if sedimentation was not complete.

Examining the pattern of the sediment at the bottom of the tubes without shaking, the readings were as follows :

Small regular disk = no agglutination = -
Granular deposit with crenated sides = +, +, +, +, +,
+, +, +, +, according to the size of the deposit.

The following controls were set up with each protocol :

Antigen (Ag) + Erythrocytes
Ag + Ab + E
Ag + C' + E
Ag + Ab + C' + E

Experiments and Preliminary Results

(1) Comparative reactivity of twelve batches of antigen :

(a) With twelve batches no haemagglutination was observed in the control with C' + erythrocytes.

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(b) The control with Ag + C' + erythrocytes gave the following pattern :

two batches : no haemagglutination = -
 six batches : haemagglutination = +
 two batches : haemagglutination = ++
 two batches : haemagglutination = +++

Such a reactivity in the control without Ab is probably due to partial sensitization of the *T. pallida* by the antibody produced by the rabbits. A similar reactivity, without addition of antibody was observed rather frequently with the regular TPIA method.

(c) With twelve batches no haemagglutination was observed in the control with Ag + Ab + erythrocytes (C' free control).

(d) Positive control with Ag + C' + Ab + erythrocytes regularly gave haemagglutination varying from ++ to ++++.

(2) Comparative reactivity of the same batch of antigen in several experiments : one batch of antigen used in six experiments gave the same figures of haemagglutination in the controls.

(3) The influence of reagent concentration was briefly studied :

Antigen, prepared as for regular TPIA, was diluted up to 1 : 4 in TPI medium without significant change in reactivity.

C' (human or guinea-pig) remained active up to a dilution of 1 : 4.

Positive sera (Ab control) produced optimal reactivity diluted (initially) 1 : 5.

Erythrocytes diluted at 2 or 1 per cent. (initially) gave good patterns.

(4) After incubation at + 5° C. no haemagglutination occurred in the positive controls.

(5) The reading was easy after 60 or 90 min. of sedimentation. After 4 to 22 hrs the patterns observed were less clear.

Sedimentation by centrifuging (5 min. at 500 r.p.m.) gave a regular disk without any pattern of haemagglutination.

(6) In a preliminary study of ten normal and ten syphilitic human sera, the haemagglutination test and regular TPIA produced identical results.

(7) Some assays were done on slides and gave good patterns of agglutination.

Discussion

The dark-field examination of the reaction mixtures, after resuspension, had shown in the controls with Ab and C' (positive control) a high proportion of *T. pallida* attached to the erythrocytes ; 40 to 60 per cent. of the organisms were adherent to the cells. Such a proportion is much greater than is observed in the regular TPIA method.

Three mechanisms of haemagglutination may be suggested :

(1) Erythrocytes with one, two, three, or four *T. pallida* attached are stopped in the sedimentation on the walls of the tube near the bottom.

(2) Erythrocytes with adherent *T. pallida* may be united through the agglutination of the *T. pallida*.

(3) Two erythrocytes may be linked by one *T. pallidum* :

Erythrocyte - C' - Ab - Ag - Ab - C' - Erythrocyte

Such a mechanism would only be concerned with the Ab responsible for the immune-adherence phenomenon.

Summary

A new method of observation of immune-adherence of *T. pallida* sensitized by specific antibody to erythrocytes in presence of C' is described. It consists in using human erythrocytes at a low concentration and is suitable for the observation of patterns of haemagglutination which are easy to read. Preliminary experiments and results are briefly reported.

REFERENCE

Nelson, R. A. (1953). *Science*, 118, 733.